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Development of a commercial vaccine for *Haemonchus contortus*, the Barber's Pole Worm

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Abstract

Quality systems were devised for making Barbervax® to the Good Manufacturing Practice standard required by the regulators, the Australian Pesticide and Veterinary Medicine Authority (APVMA). Only once this standard has been achieved do data from field trials of a vaccine count for registration purposes. A Good Manufacturing Practice licence was attained in 2011 and has since twice passed inspection by an APVMA appointed auditor, including a scaled–up version of the process.

Four field efficacy trials were subsequently conducted with lambs in New England, NSW during the summer of 2011-2012. These trials were an essential part of the work needed to obtain commercial registration of the vaccine for lambs, which was achieved on Oct 1st 2014. Two safety trials were also conducted.

Barbervax should offer the Australian sheep industry an additional, sustainable tool to control one of its most important parasites.

Executive summary

The Barber's Pole worm, *Haemonchus contortus*, is an important gastrointestinal parasite of sheep and goats in Australia and overseas. Because the parasite prefers warm moist conditions, Haemonchosis is particularly common in the summer rainfall zone especially in North Eastern NSW and Southern Qld, but the disease can occur sporadically in any State. *Haemonchus* is usually controlled by anthelmintic drugs, but strains resistant to these chemicals are common and widespread in endemic areas. Alternative methods for control are required. This report describes work which has led to Barbervax being approved for sale in lambs, as such the only vaccine in the world currently registered for any gut dwelling worm parasite of any host.

This project is the first part of a three stage objective where the overall aim is to make an effective Barber's Pole worm vaccine commercially available for Australian sheep producers. This vaccine was developed at the Moredun Research Institute in Scotland following some 20 years work and will be manufactured by one of its subsidiaries, Wormvax Australia, at the Department of Food and Agriculture, Western Australia laboratory in Albany WA .

This project was concerned with obtaining a Good Manufacturing Practice licence for the vaccine and with determining vaccine efficacy and safety for lambs in field trials. Most of the trials were done in New England, where *Haemonchus* is endemic and a serious problem (two trials not funded by MLA were also done in WA). The results were successful and culminated in the registration of Barbervax for use in lambs by the Australian Pesticide and Veterinary Medicines Authority (APVMA) on 1st October 2014.

The second project (B.AHE.0214) showed that the vaccine afforded an epidemiological benefit by reducing pasture contamination with infective worm larvae. In addition, it showed that the vaccine was effective in yearling sheep

The aim of the third project (B.AHE.0232) is to determine the ability of the vaccine to confer protection on ewes, both in terms of suppressing their increased susceptibility to infection around lambing and during lactation, an important source of infection for their lambs, but also during the high risk period in late summer when ewe deaths due to Barber's Pole worm are not uncommon.

The results have been favourable and a second dossier containing this and the yearling data is currently being reviewed by our regulatory affairs advisors with submission to the APVMA expected before the end of 2014. Details will be described separately in separate project final reports.

Therefore, once approval of the second dossier is obtained, farmers will be able to use the vaccine in lambs, yearlings and ewes. By this means it is hoped that Australian farmers will have a new tool to combat Barber's Pole worm across their entire flock.

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1 Background

The Barber's Pole worm, *Haemonchus contortus*, is an important gastrointestinal parasite of sheep and goats both in Australia and overseas. Because the parasite prefers warm moist conditions, Haemonchosis is particularly common in the summer rainfall zone especially in North Eastern NSW and Southern Qld, but fatal disease can occur in any State. *Haemonchus* is usually controlled by anthelmintic drugs, but strains resistant to these chemicals are common and widespread in endemic areas. Alternative methods for control are required, vaccination being one possibility.

After some 20 years of work, a promising *Haemonchus* vaccine called "Barbervax" has been developed at the Moredun Research Institute in Scotland. Important questions to answer were whether Barbervax would work under Australian conditions and, if so, whether it could be successfully commercialised for use in Australia and other countries.

2 Objectives

This report describes the first of three projects aimed at making an effective Barber's Pole worm vaccine commercially available for Australian sheep producers. The second and third projects will be reported separately.

This project was mainly concerned with obtaining a Good Manufacturing Practice (GMP) licence for the vaccine and with determining vaccine efficacy and safety for lambs in field trials. Most of the trials were done in New England, where *Haemonchus* is endemic and a serious problem (two trials not funded by MLA were also done in WA). The results were successful and culminated in the registration of Barbervax on 1st Oct 2014.

3 Methodology

3.1 Upscale production methods at Moredun

Six different pilot scale preparations of vaccine antigen were made, each starting from about 10g of parasites. For the scale-up preparation some 90g of worms were used. The yield of antigen per gram of parasite material, from the scaled-up preparation was in the same range as that obtained by the standard pilot-scale procedure i.e. 5.3 mg/g. (This yield has improved more than 2 fold since the project started. This was achieved by optimising various parts of the process).

3.2 Transfer scaled-up technology to Albany

Two major pieces of equipment needed for scaling up vaccine production were disassembled at Moredun, packed into a shipping container, re-assembled and commissioned in Albany.

The first of these was a machine for rapidly opening abomasa. Previously this procedure was done by hand and so this machine greatly saves on labour. The machine was tested on 500 stomachs processed over three days and performed flawlessly.

The second was a semi-automatic vaccine bottling machine, which fills and seals 250ml pillow packs. This is housed in a 6.5 m long flexible film isolator which provides a sterile atmosphere for the filling process.

3.3 Obtain GMP certificate

Three pilot scale batches of Barber's Pole vaccine were prepared successfully in Albany, W.A. using new equipment. The quality control procedure was inspected by an APVMA GMP auditor, Dr Ian Wheatley. He observed the procedures for collecting 100 *Haemonchus* infected worm donor sheep from the DAFWA farm at Mt Barker, their progress through the Albany abattoir and then the harvesting of adult worms at the Albany laboratory. He also observed the bottling machine in action when it was filling 1144 bottles with bacteriological media as a demonstration that this could be achieved aseptically.

3.4 Vaccine trials – APVMA registration application

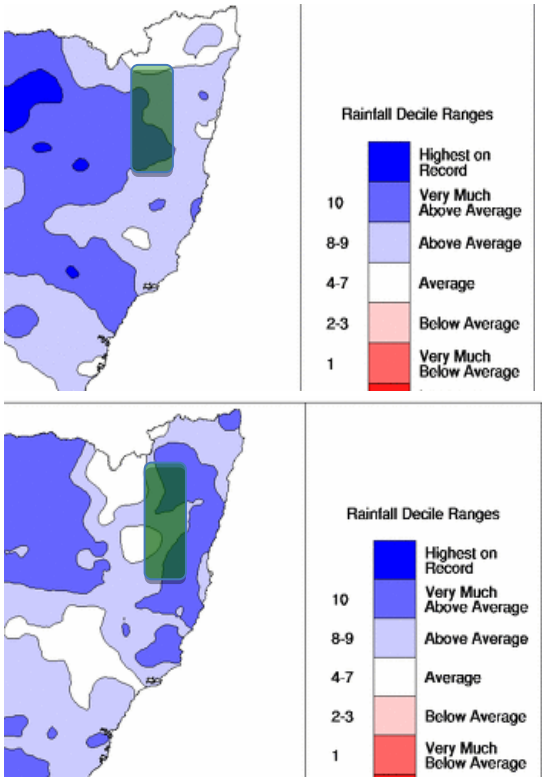
3.4.1 Efficacy trials in lambs

Three of the four efficacy trials in lambs were conducted on privately owned properties, located near Kingstown (40 mins drive SW of Armidale), Black Mountain (North of Armidale before Guyra) or Dundee (North of Glen Innes). The trials on these farms were run by Veterinary Health Research, a well-known veterinary contract research company. The fourth farm, Arding, was adjacent to the CSIRO laboratory at Chiswick and the trial was run by Dr Malcolm Knox, a highly experienced parasitologist. The trials were done in the summer of 2011-2012 and the animal phases completed in April 2012.

On all four farms the vaccine was tested in 40 lambs which grazed with 40 controls. At Arding, this main group of "Elite" Merinos (selected for fine wool) was supplemented with 40 "AB49" sheep, which had been bred to be *Haemonchus* resistant or susceptible. Ten animals from both lines were vaccinated, ten served as controls. All 120 sheep grazed together.

2011-2012 was an unusually wet spring and summer in Northern NSW, signaling an exceptionally wormy season (see rainfall maps in Figure below).

Rainfall maps of North East NSW 1st Sept 2011 to 29th Feb 2012



Spring (Sept 1 to Nov 30) Summer (Dec 1 to Feb 29)

The green rectangle contains the four NSW trial sites in New England.

Although *Haemonchus* is the dominant gut nematode species in New England and the summer rainfall zone of Australia, scouring caused by *Trichostrongylus* or *Teladorsagia* does occur, especially in wet years. Allowance was made for the control of such infections by anthelmintic treatment as the situation demanded.

Two trials not funded by MLA were also done in WA.

3.4.2 Safety trials

Two safety trials were conducted. In Trial 1, the aim was to determine whether there were any adverse local or systemic reactions when the vaccine was administered to sheep. Rectal temperatures and skin thickness at the vaccination site were measured for up to 17 days after primary or secondary vaccination.

In Trial 2, the aim was to determine whether the *Haemonchus* vaccine might adversely affect pregnancy if it was given to ewes due to lamb imminently. Twenty ewes synchronised to lamb within about a week of each other were scanned and randomly allocated to two groups of ten so that approximately the same number of lambs were

expected from each group. One group was vaccinated on two occasions one month apart, the second, control group was not vaccinated. The immunisations were timed to be approximately four weeks and one week before the predicted lambing dates. Blood samples were collected from all sheep on both vaccination days. In other words, both control and vaccinated ewes received a similar amount of handling, although the control ewes were not inoculated.

3.4.3 Registration application

The vaccine dossier has been written and subsequently reviewed and approved by the project regulatory affairs advisors. Part 1, the overview, was submitted to the APVMA and an appointment made to meet their evaluators in Canberra on March 12 2013. The vaccine project team consisted of Julie Fitzpatrick (director of Moredun Research Institute), Brown Besier (senior Parasitologist at Department of Agriculture and Food, Western Australia), Robert Dobson (parasite computer modeller, Murdoch University), Ruth Davis and Mark Albrecht, (both regulatory affairs advisors from Redcap Solutions) and David Smith (senior Parasitologist and vaccine project leader at Moredun Research Institute). The full dossier was handed in immediately after the meeting.

4 Results

4.1 Upscale production methods at Moredun

The appearance of the antigen, as obtained from both sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and native PAGE, was consistent with the appearance of the antigen prepared in Australia in both 2010 and 2011 (all six batches have been shown to protect against *H. contortus* infection).

This indicated that the process is fully scalable to production levels. 90 grams of worms produced 480 mg of antigen, the equivalent of 96,000 doses of vaccine ($\frac{480\times1000}{5} = 96{,}000$) or 384 x 250 ml pillow packs.

There was no conceivable reason why the process could not be scaled up twice more which would be about the limit it would be convenient to operate in a batch. That is, approximately 200g of parasites giving some 200,000 doses of vaccine in 800x250ml pillow packs per run.

4.2 Transfer scaled-up technology to Albany

Apart from the failure in one of the 48v power supplies in the bottling machine, all worked to plan. A temporary 24v replacement provided a solution and this will be replaced by the original part, which was not available in Australia, in due course. Once the atmosphere in the isolator was validated sterile following fumigation with H_2O_2 gas, 1,144 vaccine packs were filled with bacteriological media by the machine in three sessions. The packs were incubated at 32ºC for two weeks to determine if any were contaminated with bacteria. Only one contaminated pack was found, i.e. a failure rate of less than 0.01%

It was concluded that the large scale equipment had been transferred successfully.

4.3 Obtain GMP certificate

The three pilot scale batches of vaccine antigen passed the in-built quality control procedure which was inspected by an APVMA auditor. Apart from 7 very minor non-conformances, he could find little fault with the whole process. These non-conformances were addressed and formal written approval received. The amount of antigen made grossly exceeded the amount needed for the efficacy field trials. A 10% sample of the formulated, bottled vaccine passed a sterility test conducted at a Therapeutic Goods Administration (TGA) registered laboratory in a Perth hospital. In his annual inspection report the auditor noted 5 minor non-conformances in the GMP paperwork. These were rectified and the official clearance was received from the APVMA.

4.4 Vaccine trials – APVMA registration application

4.4.1 Efficacy trials in lambs

The results of the four efficacy trials are appended as a series of graphs (two additional trials done in the South West region of WA were not funded by MLA, but their data are included for completeness). Broadly speaking the trials worked well, with the vaccine reducing *Haemonchus* egg counts compared with control lambs on the same paddock by between 70 and 85% over the duration of each trial. No adverse effects of vaccination were reported in any of the field trials where 244 lambs were immunised with a single dose of Barbervax 4 or 5 times. The vaccine substantially suppressed *Haemonchus* egg outputs over the course of all six field trials (see Appendix 6.1). Seven of the 244 vaccinated lambs were categorised as ”non-responders”, defined as those whose counts were greater than the lower 95% confidence limit of their respective control group. Alternatively, more than 97% of the lambs responded to the vaccine and of those which did, the mean % reduction in egg count (i.e. % Protection) was 84.1% (Appendix 6.2). The equivalent figure for all sheep was 80.4%. The lambs requiring salvage treatment are shown in Appendix 6.3.

The data were compiled into detailed reports, each of which extended to more than 50 pages and formed part of the registration dossier compiled for the APVMA.

4.4.2 Safety trials

In Trial 1, a transient, but statistically significant pyrexia, measuring less than 1°C was recorded one and 3 days after the primary inoculation of vaccine and a similar increase in body temperature was recorded one day after the booster injection. Temporary pyrexia accompanied by local swelling at the inoculation site has been extensively documented for other QuilA containing vaccines currently marketed for ruminants, so it was concluded that these reactions were commercially acceptable. Starting from a mean of almost 6mm, the skin almost trebled in mean thickness by day 8 before subsiding to a mean of about 10mm from day 13 to 17 days after the first vaccination. There was considerable individual variation, however. The mean reaction seemed to be slightly less marked after the booster vaccination, though in one individual a thickness of 24mm was recorded 8 days post injection.

In Trial 2, all the ewes lambed between 33 and 41 days after they were first vaccinated and between 5 and 13 days after the second vaccination. The number of lambs born in the vaccinated group matched that predicted from scanning, but the unvaccinated ewes produced two lambs fewer than expected. Two lambs from the control group were born dead. Two of triplet lambs born to a vaccinated ewe were very weak and died a few hours after birth. Thus 16 of 18 foetuses detected in the vaccinated ewes were born and lived for at least a week whereas the same figures for the control group were 12 out of 16, a difference which is not statistically different by Fishers exact test. It was concluded that immunising heavily pregnant sheep with the *Haemonchus* vaccine was unlikely to cause abortion or affect lamb survival.

4.4.3 Registration application

Barbervax was registered for lambs by the APVMA on October 1st 2014 with the following label claim:

"A vaccine to aid in the reduction of *H. contortus* egg shedding resulting in lower pasture larval contamination and reduced disease caused by Barber's Pole worm in lambs."

5 Discussion / conclusions

Vaccine efficacy would probably have been increased if the vaccinated lambs had grazed separately, as users of the vaccine would be strongly advised to do.

A workshop was held at CSIRO Chiswick in August 2012 to discuss the findings to date with local farmers and Australian parasitology experts to elicit feedback on the vaccine's efficacy and practicality to farmers in the *Haemonchus* endemic zone of Australia. The atmosphere and feedback at the meeting were very positive and constructive, suggesting that the vaccine could be a useful additional tool.

The trial data were further given to Dr Robert Dobson, a well-known mathematical modeller, who created simulations of the epidemiological effect of the vaccine which were also presented at the workshop. The modelling indicated that the level of protection offered by the vaccine was on a par or better than existing control methods which are largely based on strategic anthelmintic treatment, especially as resistance to these drugs is increasing inexorably. It was thought that resistance to the vaccine is most unlikely to develop, making it a more sustainable method for controlling Barber's Pole.

Barbervax contains protein cleaving enzymes (H11 and H-gal-GP) normally located on the surface of the intestinal cells of *Haemonchus* where they aid in the digestion of the blood meal. Vaccinating sheep with these antigens stimulates the formation of specific antibodies which circulate in the blood of the host. When any *Haemonchus* present in a vaccinated sheep feed, they ingest these antibodies with their blood meal. The antibodies bind to and block the activity of the enzymes in the worm intestine, resulting in impaired digestion, less nutrient uptake, reduced egg output and, eventually, weakening and expulsion of worms.

These are so called "hidden" antigens, meaning that sheep naturally infected with *Haemonchus* do not recognise them immunologically, probably because the sheep

immune system is not normally exposed to proteins present on the surface of the worm intestine. The advantage of this is that *Haemonchus* has not evolved to cope with a response of this type and hence protective immunity stimulated by vaccination with these antigens works well in situations where naturally acquired immunity is weak or non-existent. The disadvantage is that, unlike with conventional antigens, the response to the hidden antigen vaccine is not boosted by exposure to the parasite. It can only be maintained by further injections of the vaccine.

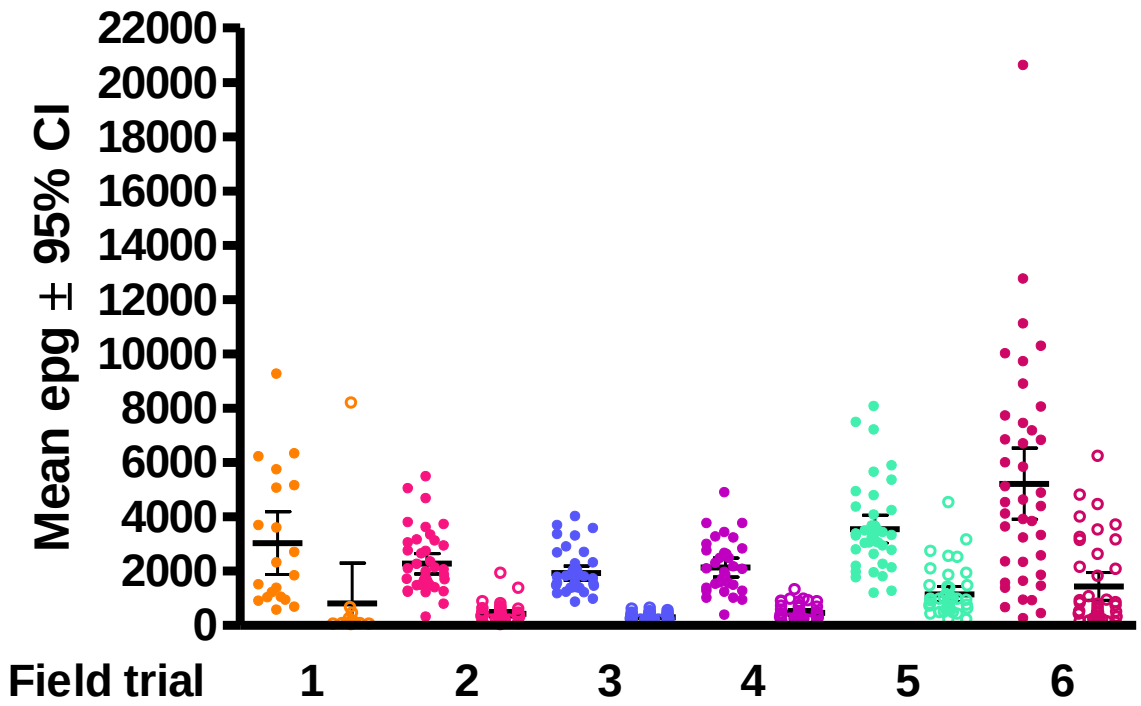
It has been shown experimentally that vaccination with H11 does not interfere with the development of the natural immunity which is slowly acquired by sheep when they are continuously exposed to *Haemonchus* larvae, as occurs on pasture. Rather, vaccine and natural immunity provide separate, complementary forms of protection to the sheep.

6 Appendix

6.1 Mean egg counts

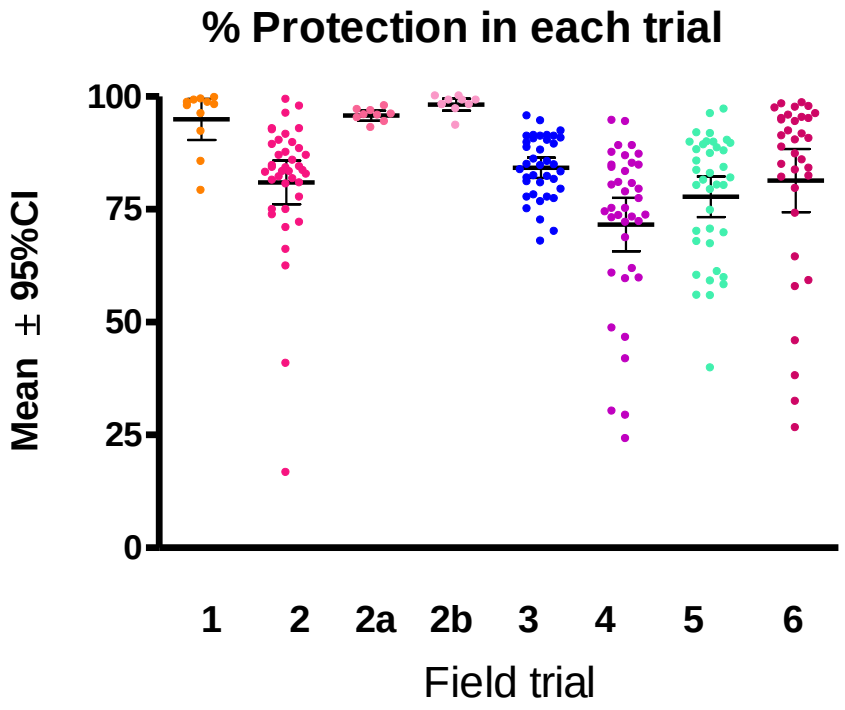
Mean egg counts (and 95% confidence intervals) of all vaccinated and control sheep over the course of each trial are shown in the figure below. Sheep in the same trial share the same colour circles; filled circles = controls, open circles = vaccinates.

For Field trial 1 only the group vaccinated with 5µg antigen given 6 weeks apart are plotted and for Field trial 2, only the Elites are shown as the counts of the AB49s are too low to be visualized on this scale of graph. The vaccine substantially suppressed *Haemonchus* egg outputs over the course of all six Field trials. Trials 1 & 6 done in WA were not funded by MLA, but their data are included for completeness.



6.2 Reduction of egg output

Mean % protection (% reduction of egg output over the trial compared to respective controls) for each of 6 field trials with Barbervax are shown below. Seven of the 244 vaccinated lambs were categorised as "non-responders", defined as those whose counts were greater than the lower 95% confidence limit of their respective control group. Alternatively, more than 97% of the lambs responded to the vaccine and of those which did, the mean % reduction in egg count (i.e. % Protection) was 84.1%.The data from 7 non-responders have been omitted from the graph. Field trial 1 shows the data from the 5µg, 6wk group only. Field trial 2 shows the Elite line data only, 2a and 2b show the susceptible and resistant AB49 lines, respectively. Trials 1 & 6 done in WA were not funded by MLA, but their data are included for completeness.



6.3 Salvage treatments required

The total number of vaccinated and control lambs in the six field trials requiring "salvage" treatment is shown below. Many lambs, especially the controls, were treated more than once. Sheep in the same trial share the same column colour; filled columns = vaccinates; empty columns = controls. Field trial 2 shows the Elite line data only, 2a and 2b show the susceptible and resistant lines, respectively. It can be seen that in every trial, the vaccinated lambs required fewer treatments than their respective controls, clearly showing that Barbervax was reducing the degree of anaemia caused by *Haemonchus.* Trials 1 & 6 done in WA were not funded by MLA, but their data are included for completeness.